Physicochemical properties, FTIR spectra, scanning electron microscopy and functional properties of protein isolate produced from wild apricot kernel press cake

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Abstract

Protein isolates were obtained from defatted wild apricot press cake by isoelectric precipitation. The physico-chemical and functional properties of wild apricot protein isolate was significantly different. The wild apricot protein isolate had moisture content 12.67 per cent, ash content 5.84 per cent, crude protein 90.15 per cent, soluble per cent 14.65 per cent, crude fibre 0.21 per cent and crude fat 0.95 per cent. Whereas, colour lightness (L *) value 22.01, redness (a*) value 6.45 and yellowness (b*) value 17.85, respectively. The FTIR spectra of wild apricot protein isolate was basically indistinguishable in the wave-number range of 4000–400 cm^{-1} indicate the presence of amide II and this amide showed the protein units and most prominent vibration bands of the protein backbone. Protein isolate had some cracked and big flaky plate like structure surface represents the presence of protein and indicate the presence of more protein content in the sample. Wild apricot protein isolate had higher water absorption, oil absorption capacity and higher protein solubility (2.45 ml/g, 2.52 ml/g and 88.00%). These results demonstrate that wild apricot protein isolate is a suitable ingredient for the development of different value added products.

Keywords: Wild apricot protein isolate, isoelectric precipitation, functional properties

Introduction

Proteins are the principle structural and functional component of many food systems e.g. meat, cheese, egg white, legumes and many cereals products. Protein is derived from a greek word meaning first or primary because of the fundamental role of proteins in sustaining life. Protein plays a functional role not only in sustaining life, but also in foods derived from plants and animals. Proteins are of various origins and can be group into animal proteins (gelatin), vegetable proteins (soya bean, peanut protein, apricot protein and wheat protein) and animal-derived proteins (milk proteins). Among these proteins, plant based (vegetable protein) proteins have attracted a great interest as a source of low-cost protein to supplement human diets and have acceptable functional properties (Garba and Kaur, 2014). Plant derived proteins are the good source of proteins, which have been used as a substitute for meat and play a significant role in alleviating protein-energy malnutrition. So isolation of proteins from different plant sources, has gaining the attention to isolate the protein from different plant sources.

Protein isolates are the refined forms of protein, containing the greater amount of protein with greater digestibility. Protein isolates are the acceptable ingredient for food application, due to their fine particle size, dispersibility, emulsification and emulsion stability. Protein isolates developed from a variety of plant sources

viz. soyabean, peanut, cashewnut, almond, apricot, seasame and many beans. Oilseeds are valuable sources of lipid, oils and proteins. So after extraction of oils from oilseeds, the press cake can be better alternative for protein isolates which contains higher amount of proteins. Wild apricot is an important temperature fruit and commercially cultivated in different parts of the world and in India, it is mainly grown in Himachal Pradesh, Jammu and Kashmir, Uttarakhad and to a limited extent in the Nilgiries with an annual production of 18,999 tonnes from an area of 5000 ha (FAO, 2015). The wild apricot fruit due to their perishable nature are used as fresh and are also used for preparation of different value added products. The aim of the present study was to evaluate the physicochemical and functional properties of isolated protein from apricot press cake to improve its nutritionally and the ability its use as a food supplement.

Materials and Methods

Procurement of raw materials

Wild apricot press cake was procured from the Department of Food Science and Technology Solan. Whereas other materials purchased from the Solan market (HP) and brought to the laboratory of Department of Food Science and Technology for the further studies.

Process for preparation of protein isolate by isoelectric precipitation

The defatted wild apricot press cake mixed with the distilled water at ratio of 1:20 and adjusted the pH using 0.1 N NaOH for maximum solubilization of protein. The slurry was stirred in water bath for half an hour at 20-25^{0}C temperature and then centrifuged at 4000 rpm for 20 min. The pH of the supernatant was adjusted at its isoelectric point (4.0) using saturated citric acid solution to precipitate the protein. The precipitated protein collected and kept in dehumidifier and store at ambient temperature for further use.

Physicochemical properties analysis

The chemical composition in terms of moisture, ash, fat, crude fibre and protein was determined as described by AOAC, (2000). The protein contents of the raw materials and protein isolates were calculated based on nitrogen content (N% X 6.25). Whereas, Colour of samples was measured in a Lovibond Colour Tintometer Model PFX-I series spectrocolourimeter in which RYBN colour units were obtained along with CIE readings i.e. L^* a^* and b^* values.

Fourier Transforms Infrared (FTIR) Spectroscopy analysis

For the qualitative analysis of different samples subjected to FTIR analysis (Shimadzu 8400S FTIR spectrometer, equipped with KBr beam splitter) using approximately 5 mg of each sample along with 5 mg KBr. FTIR spectrophotometer was operated at a spectral range of 4000–400 cm^{-1} with a maximum resolution of -0.85 cm^{-1}. The spectra so obtained for the respective samples were interpreted as per the guidelines given by Stuart (2004).

Microstructure (SEM)

The morphology of sample was evaluated using a EmCraft (Korea): Table-top scanning electron microscope (SEM Cube-1000). Samples were dehydrated by putting them into critical point drying equipment or freeze dried. The mucilage powder was fixed in an aluminum plate (specimen holder), using an electrically conductive tap and a coating of gold at 10 mbar for 90 s was applied. Each sample was transferred to microscope for observation. The procedure was applied to gain information about the arrangements of particle that correlated with structure of samples. The microscope was operated at 5 kV and different levels of magnification: 1000X and 1200X

Functional properties analysis

Water Absorption Capacity

Water absorption capacity (WAC) was determined by dissolving 0.5 g of the sample with 10 ml of distilled water in centrifuge tubes and vortexed for 30 s. The dispersions were allowed to stand at room temperature for 30 min, centrifuged at 3000 rpm for 25 min. The supernatant was filtered with Whatman No 1 filter paper and the volume retrieved was accurately measured. The difference between initial volumes of distilled water added to the sample and the volume obtained after filtration was calculated. The results were reported as mL of water absorbed per gram of sample.

Oil absorption capacity

Oil absorption capacity (OAC) was estimated by taking 1 g of the sample (W_0) into pre-weighed 15 ml centrifuge tubes. Mix thoroughly with 10 ml (V_1) of refined pure groundnut oil by using vortex mixer. Samples were allowed to stand for 30 min. The sample-oil mixture was centrifuged at 3000 rpm for 20 min. Immediately after centrifugation, the supernatant was carefully poured into a 10 ml graduated cylinder, and the volume was recorded (V_2).

$$\text{Oil Absorption Capacity} = \frac{V_1 - V_2}{W_0}$$

Emulsification capacity/activity

To determine the emulsifying activity, sample (1-5g) is homogenized for 30 seconds in 50ml water using homogenizer at approximately 10,000rpm then corn oil (25ml) added to the mixture and again homogenized for 30 sec. The emulsion is divided into two equal volume aliquots and centrifuged at 1100 rpm for 5 min, the aliquots is heated for 15 min at 85^{o}C. The ratio of the height of emulsion to the high of liquid layer is noted to calculate emulsion activity.

Foaming capacity/activity

The capacity of foams can be determined by dispersing 50ml of 3 per cent (w/v) of sample in distilled water and transfer immediately into a graduated cylinder, the volume should be recorded before and after whipping. The foaming capacity can be expressed as the percentage volume induced by whipping.

Protein solubility

To determine the protein solubility, the sample (0.5g) should be homogenized in 20 ml of 0.1 M NaCl at pH of 7.0 for 1h followed by centrifugation at 10,000 rpm for 30 min. Nitrogen content be determined in the soluble fraction and the solubility can be expressed as the percentage total nitrogen of the original sample to that of soluble fraction.

Statistical analysis

All the analytical parameters were recorded in triplicates and the mean value of each parameter was described. The data pertaining to the extraction of protein isolate was analyzed by the response surface methodology. The data of quantitative estimation of biochemical characteristics were assessed by factorial CRD whereas the data pertaining to sensory evaluation were analyzed by RBD using two factors analysis of variance (AOVA) with the help of OPSTAT software (Cochran and Cox 1967).

Results and Discussion

Proximate composition of protein isolate

The proximate compositions of wild apricot protein isolate are presented in Table 1. The moisture content of the isolate was 12.67 per cent. Whereas, the water activity was 0.88. Total ash, crude protein, soluble protein, crude fibre and crude fat were recorded to be 5.84 per cent, 90.15 per cent, 14.65 per cent, 0.21 per cent and 0.95 per cent respectively. The results were in conformity with the finding of Sharma *et al*. (2010). They reported that apricot protein isolate contained 9.10 per cent moisture, 0.8 per cent ash, 68.80 per cent crude protein, 6.4 per cent crude fat, 2.2 per cent crude fibre and 0.00 mg/100g HCN. Ulloa *et al*., (2017) had reported that jackfruit seed protein isolate contained 0.8 (g/kg) fat, 10.30 (g/kg) ash and 844.3 (g/kg) protein. Similar results were reported by Nicanor *et al*., (2014) in mamey sapote protein isolate that contained 13.00 (g/kg) fat, 15.00 (g/kg) ash, 21.00 (g/kg) crude fibre and 950.90 (g/kg) protein.

Table 1Physicochemical properties of Protein isolate

Parameters	**Observations (Mean ± SE)**
Moisture (%)	12.67 ± 0.02
Water activity	0.88 ± 0.01
Total ash (%)	5.84 ± 0.02
Crude protein (%)	90.15 ± 0.03
Soluble protein (%)	14.65 ± 0.03
Crude fibre (%)	0.21 ± 0.01
Crude fat (%)	0.95 ± 0.03

Colour value of protein isolate

Colour is very important parameter in judging the properties of suitable raw material used for the preparation but also provides information about the formulation and quality of the end product. The colour values L*, a * and b * of protein isolate are shown in Table 4.9. The protein isolate has lightness (L *) value 22.01, redness (a*) value 6.45 and yellowness (b*) value 17.85. Mugendi *et al*. (2010) had reported similar results in mucunna bean protein isolate and observed that lightness (L *), redness (a*) and yellowness (b*) were 36.39, 0.47 and 8.30, respectively. Whereas, Alabi and Falade. (2017) had reported lightness (L *) 89.84, redness (a*) 0.95 and yellowness (b*) 15.27 in groundnut protein isolate.

Table 2 Colour properties (Lab) of protein isolate

Properties	value
L * (Lightness)	22.01
a * (Redness-greeness)	6.45
b * (Yelloeness- blueness)	17.85

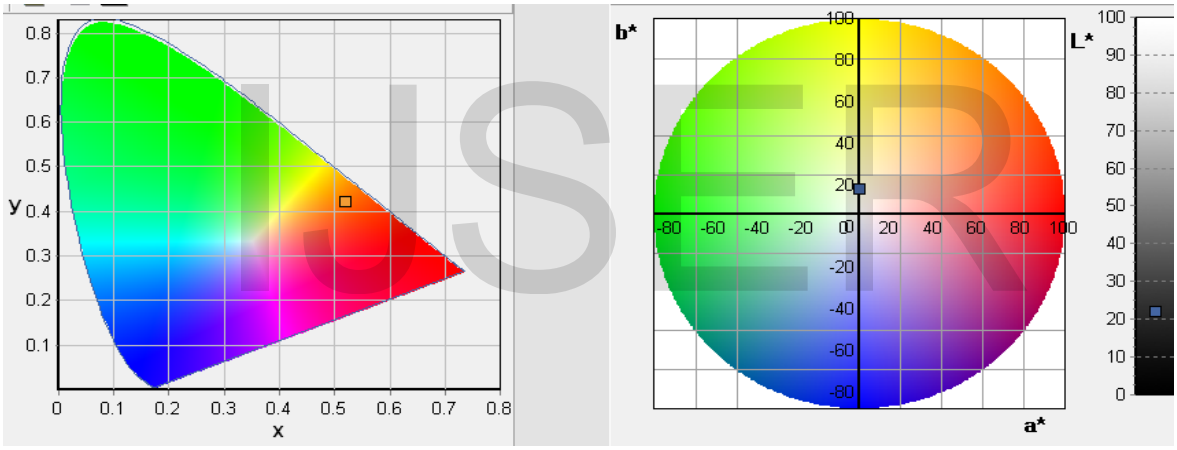

Fig 1 CIE readings of protein isolate

FTIR spectra of protein isolate

Table 3 FTIR frequencies and their peak assignments for the spectra

Apricot protein isolate		
Peak	**Area**	**Compounds**
441.71	18.70	P-S stretching
522.73	26.70	P-Cl stretching
1047.38	35.50	C-C stretch of starch
1456.30	13.90	Methylene scissory
1541.18	21.70	Secondary amide N-H bonding
1651.12	17.90	C= C stretch, C=O stretch
2011.82	12.80	Combination N-H stretching, Combination O-H stretching
2156.49	25.20	Aliphatic isonitrile –N=C stretching
2924.18	47.60	O-H stretching (Carboxylic acid)
3751.67	18.70	O-H stretching (Water)

FTIR has been used to analyze varieties of samples due to its ability to identify functional group of chemical compounds, such as carbohydrate and ester, as well as inter atom chemical bonds. FTIR has high accuracy level in identifying process (Smith, 1979). The FTIR spectra of apricot protein isolate was basically indistinguishable in the wave-number range of 4000–400 cm^{-1}, only with subtle differences in the intensity of bands/peaks (Fig 2). Infrared spectra of apricot protein isolate indicated by the presence of broad band at 2924.18 cm^{-1}, which attribute to O-H stretching vibrations. This is due to carboxylic compounds in the polymer protein isolate matrix. Combination of N-H stretching at 2011.82 cm^{-1} showed the presence of amine functional group as indicative of protein content. Whereas, the absorption band at 1541.18 cm^{-1} assigned to amide II and this amide showed the protein units and most prominent vibration bands of the protein backbone. High sensitivity to small variations in molecular geometry and hydrogen bonding patterns makes the amide band useful for protein structural composition. Widjanarko *et al.* (2010) had studied the FTIR spectra of corn protein isolate (CPI) and wheat protein isolate (WPI) and reported that presence of carboxylic and amide compounds at CPI and WPI at bands intensity of 3235.37 cm^{-1} and 3432.09 cm^{-1}, respectively. Whereas peak at 1635.52 cm^{-1} region, 1647.1 cm^{-1} and 1634.56 cm^{-1} regions, respectively indicated the presence of functional compounds of protein amide groups –CONH– which were attributed to carbonyl (C=O) stretch vibration. They also observed that native protein gave more intense peaks in the range of 1630 - 1660 cm^{-1}, meaning that several overlapping secondary structural components of the polypeptide chains were present in the protein. Wen *et al*. (2009) reported that the absorption bands at 3435.95 and 3488.99 cm^{-1} were assigned to –OH and NH_2 stretch vibration, indicating the presence of overlapping –OH groups of the carboxylic groups and –NH_2 of amide groups in the mixtures of protein isolates matrix.

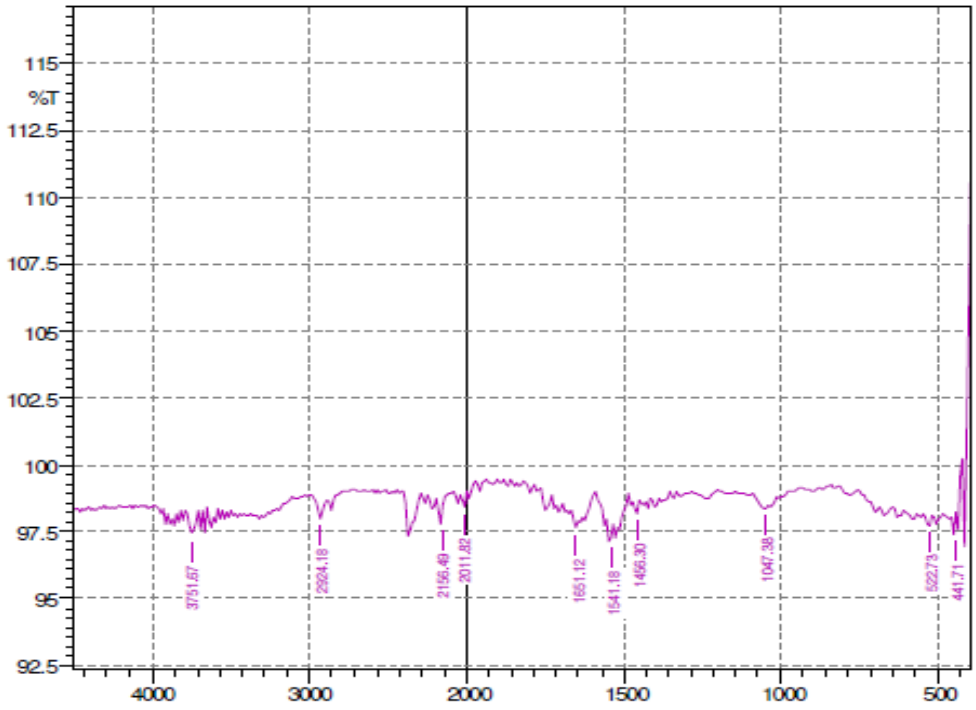

Fig 2 FTIR spectra along with wavelength of the wild apricot protein isolate

Scanning Electron Microscopy (SEM) of protein isolate

The microstructure examination is conducted to study the magnification under scanning electron microscopy (SEM) to determine the microstructure, chemical composition and physical (size and shape) characterizations. The objective of the microstructure analysis is to elucidate the relationships between protein and starch matrix in the food structure (Autio and Laurikainen, 1997). The SEM was conducted at magnification of 1000 x and 1200x. The Fig 3 revealed that, the isolate had cracked and big flaky plate like structure surface represented the presence of protein and indicated the presence of more protein content in the sample. It was suggested that big flaky plate like structure of protein isolate further contributes to protein solubility of the isolate. Similar flaky structure of protein isolates were reported by Mao and Hua (2012) in walnut protein isolate and Kaptso *et al*. (2014) in groundnut protein isolate.

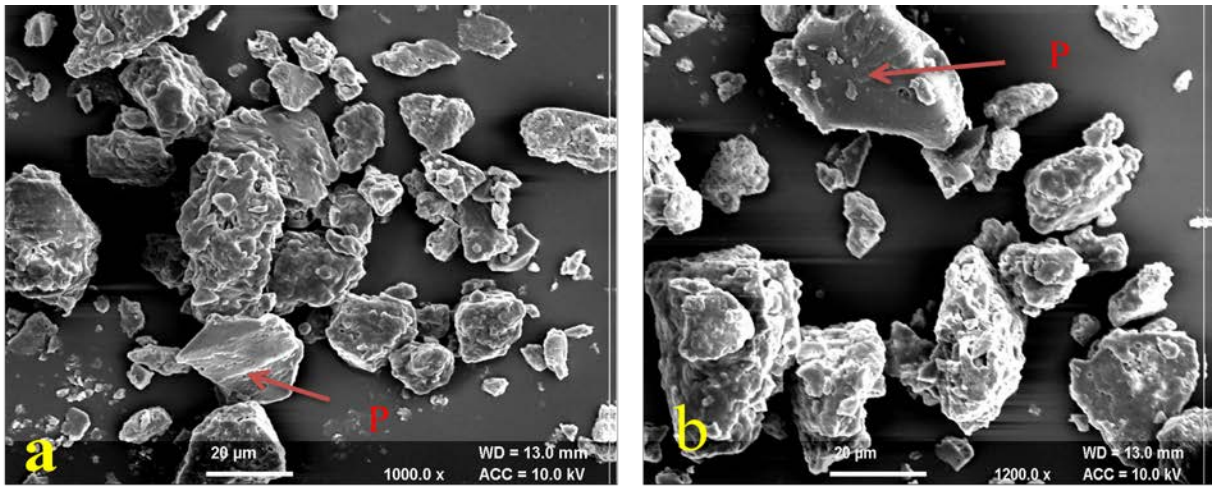

Fig 3: Scanning electron microscopy (SEM) of wild apricot kernel press cake protein isolate: (a) magnification 1000x, (b) magnification 1200 x: P- Protein

Functional properties of protein isolate

The functional properties of a protein are those physical and chemical properties, which affect the behavior of proteins in food systems during storage, processing, preparation and consumption.

Table 4. Functional properties of protein isolates

Parameters	Observation (Mean±SE)
Water absorption Capacity (g/g)	2.45 ± 0.20
Oil absorption Capacity (g/g)	2.52 ± 0.10
Emulsion activity/Capacity (%)	52.00 ± 1.10
Protein solubility (%)	88.00 ± 2.25
Foaming Capacity (%)	20.00 ± 0.30

Water absorption capacity (WAC) and oil absorption capacity (OAC) of food materials is an important functional properties because it improves mouthfeel and flavour retention of food. Protein has both hydrophilic and hydrophobic properties therefore, can interact with water and oil in foods. Whereas, High water absorption of protein isolates help to reduce moisture loss in package products (Butt and Batool 2010). Protein isolate has water absorption capacity (WAC) of 2.45ml/g, Oil absorption capacity (OAC) of 2.52ml/g. Our results are confirmatory with the earlier findings of Ogunwolu *et al*. (2009), Quinela *et al*., (1997), Sharma *et al*., (2010) and Tounkara *et al*., (2013) in Cashewnut protein isolate, faba bean protein isolate, apricot protein isolate and Roselle seed protein isolate, respectively. Protein isolate has emulsifying activity/capacity of 52.00 per cent. Our observations also agreed with earlier findings of Ulloa *et al*., (2017) and Abbas *et al*., (2015) in jackfruit seed protein isolate (52.00%) and defatted peanut flour protein isolate (52.15%). Whereas, protein solubility relates to the surface hydrophobic (protein-protein) interaction and hydrophilic (protein-solvent) interactions with water. Protein isolate has Protein solubility of 88.00 per cent. Our results are confirmatory

with the findings of Quintela *et al*., (1997), Ogunwolu *et al*., (2009) and Ulloa *et al*., (2017) in soybean protein isolate (85.00%), cashewnut protein isolate (80.00-96.00%) and jackfruit seed protein isolate (70.00-80.00%), respectively. Protein isolate has foaming capacity of 20.00 per cent. Similar results were reported by Quinela *et al*., (1997) and Sharma *et al*., (2010) in soybean protein isolate (22.00%) and apricot protein isolate (21.00%).

Conclusion

Wild apricot press cake, a by-product of the fruit industry, is a potential raw material for the production of a protein isolate when subjected to isoelectric precipitation method. The wild apricot protein isolate showed good physicochemical properties as protein, fibre, fat and also showed good FTIR spectra which indicate the presence of protein and amide content in the isolate. Protein isolate also showed good functional properties in term of its water and oil binding capacity, protein solubility and emulsification and foaming capacity. Therefore, apricot protein isolate could be a novel protein source applied in food system.

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